STUDIES IN THE BIOLOGY OF THE LEECH. II.

Responses of the Leech to Electrical Stimulation.

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INTRODUCTION.

Direct current of low voltage has proven to be an excellent stimulus of motor neurons when the animal can be completely immersed in water. By this means it is possible to effect a direct motor neuron stimulus or motor neuron depression in those neurons which lie parallel to the current. The direct current acts as a constant force which results in the production of a prolonged response. (Loeb, Lillie, Shensa and Barrows.) It provides a uniform stimulus, the intensity of which may be changed at the will of the investigator to best serve his needs.

The following group of experiments will illustrate the effectiveness of this type of electrical stimulation.

ANTERIOR POSTERIOR STIMULATION.

The leech[1] to be used in this experiment was placed in a glass tray the bottom of which was kept moist. Movable copper electrodes were placed in the tray in contact with the moist surface of the tray. A direct current of 18 volts was used. A reversing switch was connected in the circuit.

The leech was so arranged in the tray that the positive electrode was behind the posterior end of the animal and the negative electrode was in front of the anterior end. When the current was turned on the leech extended to a length surpassing that of normal extension. It maintained this prolonged extension and crawled toward the negative electrode.

When the poles were reversed, that is when the positive electrode was in front of the anterior end and the negative electrode behind the posterior end the leech contracted, and irregular movements of the anterior and posterior ends were pronounced. The anterior end often raised itself from the surface of the container. Following random movements, of the

[1]The leeches used in these experiments were all *Haemopis marmoratis* (Say).

above described nature, the anterior end turned and crawled toward the negative electrode.

Direct current, 18 volts.
The normal length of the leech extended..........14 cm.
The normal length of the leech contracted........ 5 cm.
The average length of the leech.................. 7 cm.
With the negative pole at the anterior end the leech extends to a length of........................16 cm.
With the negative pole at the posterior end the length of the leech is......................... 7 cm.

A similar experiment to the one above was conducted on the earthworm. The results in both cases were identical. Both worms elongated when the negative electrode was placed in front of the anterior end and both shortened when the negative electrode was placed behind the posterior end.

An explanation of this phenomenon will be given in a later issue of this Journal.

DORSAL VENTRAL STIMULATION.

A zinc plate was placed in the bottom of a shallow glass container. This container was filled with tap water. Near the surface of the water was fastened a galvanized iron screen. This screen was placed an inch to an inch and one-half above the zinc plate. A reversing switch was connected in the circuit. Three dry cells supplied the current.

A leech was placed in the container and came to rest on the zinc plate. With the positive charge on the zinc plate and the negative charge on the wire screen the following reaction occurred:

The leech remained with its mid-ventral surface in contact with the zinc plate. The anterior and posterior ends of the leech were raised above the surface of the plate extending toward the wire screen. The middle region of the leech was contracted. The anterior and posterior ends were extended.

When the poles were reversed, i. e., the negative current connected to the zinc plate and the positive connected to the wire screen, the following reaction occurred:

The leech turned over bringing its dorsal surface in contact with the zinc plate. The leech may roll to right or left, even turning completely over, but eventually it comes to rest with the ventral surface toward the wire screen (positive electrode).

The anterior and posterior ends remain in contact with the zinc plate (negative).

When an earthworm was placed under circumstances similar to that of the leech in the preceding experiment, a somewhat different response was noted. In the first place there did not seem to be a definite orientation to the current. The earthworm rolled over and over, contracting violently at times. This difference in behavior may be explained on the basis of the difference in the regions of concentration of the subepidermal plexus in the two animals. Recent reports from the behavior laboratory (unpublished) give for the earthworm lateral concentrations of the subepidermal plexus. When this mechanism in the earthworm is compared with that in the leech there is some basis for explaining the differences in behavior. As stated in a previous paper there are four concentrations of the subepidermal plexus in the leech, two dorso-lateral and two ventral-lateral. (Miller, 1933.)

The response to dorso-ventral electrical stimulation of the leech was a direct orientation of the animal. The negative current above the dorsal surface directly stimulated the dorsal longitudinal muscle fibers through the subepidermal plexus. The ventral series of longitudinal muscles were inhibited. Through direct motor stimulation in the anterior and posterior ends the circular muscles at the extremities were contracted. This resulted in the leech assuming a U-shaped position.

With the ventral surface of the leech in contact with the zinc plate to which had been attached the negative electrode the longitudinal series of ventral muscles contracted. This contraction resulted in the animal assuming the temporary position like that of an inverted "U." As the leech rolled over, bringing the dorsal surface in contact with the zinc plate, the dorsal series of longitudinal muscles contracted raising the center of the leech toward the wire screen. Throughout this experiment the dorsal surface of the leech has been oriented toward the negative. The anterior and posterior ends had been extended toward the negative. This orientation is the result of direct neuro-motor stimulation on the muscles of the body wall through the subepidermal plexus.

Lateral Stimulation

The bottom of a shallow glass tray was covered with tap water. In this tray was placed a leech. The positive electrode was placed to the right of the mid-body region. The negative electrode was placed in a similar position on the opposite side of the animal. These electrodes were placed several inches from the edge of the leech and in contact with the water in the container.

Upon the application of the current the leech twisted and rolled. Very shortly after the initial shock the anterior end turned toward the negative pole. The posterior end raised from the surface twisted and turned toward the negative pole. When the poles were reversed the same reaction occurred on the opposite side.

A similar phenomenon to that described above has been known for some time to take place in the earthworm. Of the explanations that have been advanced to account for this behavior, that of Shensa and Barrows (1932) is the most reasonable to date.

The following experiment conducted with leech material is essentially the same as that described by Shensa and Barrows for the earthworm. In this report I will not repeat details of the experiment, as these have been previously reported by the above authors. I will, however, briefly explain the principle of the experiment, the results and the application to the explanation of the leech behavior.

The purpose of this experiment was to determine the relationship between the subepidermal nerve plexus and the lateral muscular contraction of the leech following lateral electrical stimulation.

A strip of the lateral body wall of the leech was removed. This strip of tissue consisted of the epidermis and muscles of the circular and longitudinal series, but did not contain any part of the ventral nerve cord. This strip was suspended in a tumbler of tap water. On each side of the strip were suspended parallel strips of copper screening which served as electrodes. The lower end of the strip of tissue was fastened to a stationary arm, the upper end to a movable writing arm of a kymograph lever. A lever registering time in seconds and another to indicate the current change were placed against the smoked paper of the kymograph drum.

With the negative current applied on the skin side and the positive current on the muscle side, the strip of tissue contracted. Reversing the poles of the electrodes the strip of tissue showed a gradual relaxation.

The same strip of tissue was treated with novocaine after which it was attached and stimulated as before. No muscular contraction could be detected. The same narcotised strip when stimulated directly, that is by attaching a fine copper wire to both ends, responded by definite contraction.

I have shown in the above experiment that the subepidermal nerve plexus exercises the same control over the circular and longitudinal muscles as previously demonstrated for the earthworm, and that this phenomenon explains the behavior of the leech to lateral electrical stimulation.[2] It gives definite evidence of the role played by the subepidermal nerve plexus in annelid behavior.

SUMMARY.

1. Anterior, posterior, and lateral electrical stimulation of the leech and earthworm resulted in identical reactions.
2. Dorsal ventral electrical stimulation of the leech and the earthworm resulted in quite different responses.
3. These differences in behavior are explained on the basis of neuro-muscular differences between the two types of animals.
4. Dorsal ventral electrical stimulation results in a definite orientation in the leech.
5. This orientation is the result of direct motor stimulation (i. e., without intervention of neurons in the nerve cord) on the muscles of the body wall through the subepidermal nerve plexus.
6. Experimental evidence obtained here substantiates the postulated polarity of motor neurons in the leech.

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[2] A detailed explanation of the influence of electrical stimulation and an explanation of certain phases of leech behavior will appear in a later issue of this Journal.